**Degradation Parts**

Lignocellulose is composed of cellulose, hemicellulose, and lignin. The fermentation of lignocellulose biomass to ethanol is an attractive route to fuels that supplements the fossil fuels. However, one barrier to the production of ethanol from biomass is that the sugars necessary for fermentation are trapped inside the lignocellulose. To extract the fermentable sugars, we must first disconnect the celluloses from the lignin, and then break the newly freed celluloses down into simple monosaccharides.

**Method:** We have planned to construct two whole-cell biocatalysts with the ability to induce synergetic and sequential cellulose and hemicellulose degradation reactions through co-display of two types of degradation enzymes on each of the Pichia pastoris cell-surface.

In order to make sure that several of our cell display enzymes are actually displayed on the surface of yeast, we utilized Immunofluorescence technique and Flow Cytometry to detect protein location and their relative abundance.

**Result:** We constructed several strains of Pichia pastoris that pretreat lignocellulose, an important biomass resource, degrade cellulose, hemicellulose, and lignin, the three polymers of lignocellulose, and finally generate ethanol.

We have primarily constructed a pathway that incorporates enzymatic hydrolysis and bioethanol production. We have successfully engineered four different routes for lignin degradation.

**From Lignocellulose to Biofuel: A synthetic biology approach**

Lignocellulosic biomass to ethanol

**Biocatalyst**

Lignocellulose → Glucose → Ethanol

**We constructed three strains of Pichia pastoris that pretreat lignocellulose, an important biomass resource, degrade cellulose, hemicellulose, and lignin, the three polymers of lignocellulose, and finally generate ethanol.

**Biocatalyst**

Lignocellulosic biomass to ethanol

**We constructed several strains of Pichia pastoris that pretreat lignocellulose, an important biomass resource, degrade cellulose, hemicellulose, and lignin, the three polymers of lignocellulose, and finally generate ethanol.**

**Future Work**

1. We have tried to use enzyme assay methods to determine the enzymes activities, but we didn’t get satisfying results. We will find more effective methods later, to ensure that those enzymes activities can be accurately determined.
2. We will explore the optimum condition for yeast fermentation.
3. We would like to analyze the synergistic reaction of the cellulases and hemicellulases codisplayed on the cell surface and to construct a yeast whole-cell biocatalyst with an improved ability to catalyze cellulose and hemicellulose degradation and fermentation.

**QS Model & BF model**

**Quorum Sensor Model**

In order to control the reaction rate of substrates and speed of bacteria’s growth, we incorporate a pathway containing CsgD which can be used to induce cell density. X, represented as density, can be regulated by concentration of CcdB and CsgD. The equation of CsgD concentration as in any other traditional practice in the water treatment.

**Biofilm-Formation Model**

With the chemotaxis of E. coli, the oxygen-concentration near the cathode is lower and lower. Then the oxygen-sensitive promoter Pvgd and the promoter PureI are activated by hydrogen ions and micro-aerobic atmosphere which lead to the formation of CsgD. Our quorum Senor Model can be activated by hydrogen ions and micro-aerobic atmosphere which lead to the formation of CsgD. The ODs for the strains involved in the biofilm-formation are given below:

**CDC Model & OC Model**

**Cell-Density Control Model**

In order to control the reaction rate of substrates and speed of bacteria’s growth, we incorporate a pathway containing CsgD which can be used to induce cell density. X, represented as density, can be regulated by concentration of CsgD and CsgD.

**Oxygen Concentration Model**

In the research that treating sewage by means of MFCs to generate electricity, researchers found that the capacity of electricity generation and production greatly enhanced after the formation of biofilm, compared to a single free bacteria microorganism, which inspires us to generate electricity after making MFCs to produce biofilm. Here, in our research, we design four devices to conduct our biofuel generation process, including the chemotaxis to Hydrogen ions’ concentration gradient, the formation of biofilm, cell density control and process of bioelectrogenesis.

**Biocatalysis**

Lignin Degradation

**Biofilm Formation**

The equation can be solved as below:

\[
K_c \left( C_{Cd} - C_{Cd}^0 \right) = \left( C_{Cd} - C_{Cd}^0 \right) = \left( C_{Cd} - C_{Cd}^0 \right)
\]

**QS Model**

\[
\text{OD}_{Pvgd} = \frac{\left( C_{Cd} - C_{Cd}^0 \right)}{K_c}
\]

**BF model**

\[
\text{OD}_{PureI} = \frac{\left( C_{Cd} - C_{Cd}^0 \right)}{K_c}
\]